which separated on cooling melted at 200° after recrystallization from methanol. The m.p. was not depressed by mixing with a specimen of bis-(2-hydroxy-1-naphthyl)-methane (lit.[14] m.p. 200°); yield 84%.

Rearrangement of III to II. **Procedure A.**—A solution of 2 g. of N,N-bis-(2-naphthoxymethyl)-amine (III) in 40 ml. of 2-ethoxyethanol containing 4 g. of boric acid was heated under reflux for two hours. The cooled reaction mixture was poured slowly into 150 ml. of cold water and the resulting precipitate (1.67 g., 83%) removed by filtration; m.p. 178–190°, after recrystallization from acetone–water.[13]

Acetylation of the product at room temperature with acetic anhydride gave a derivative melting at 139–141° after recrystallization from methanol–water. The m.p. was not depressed when the derivative was mixed with an authentic specimen of VI.

Treatment of the acetyl derivative (m.p. 139–141°) with sodium hydroxide in methanol at room temperature followed by acidification gave a solid (69% yield) melting at 194–196° after recrystallization from methanol–water. The m.p. was not depressed when the product was mixed with an authentic specimen of V.

Procedure B.—A solution of 2 g. of N,N-bis-(2-naphthoxymethyl)-amine (III) in 50 ml. of butanol-1 containing 5 ml. of concentrated hydrochloric acid was heated under reflux for three hours. The crystalline product obtained by concentration and cooling of the reaction mixture was removed by filtration and washed with acetone and ether; m.p. 197–204°; yield 1.5 g. (68%). A solution of the product (0.85 g.) in 25 ml. of methanol and 10 ml. of pyridine was added to 150 ml. of water. The resulting solid (0.55 g.) was removed by filtration and washed successively with water, ethanol, and ether in that order; m.p. 141–145°.[13] The m.p. was not depressed when the product was mixed with a sample of N,N-bis-(2-hydroxy-1-naphthylmethyl)-amine (II) prepared by reduction of I.

Treatment of the product with acetic anhydride in pyridine gave a solid (m.p. 139–141°) which did not depress the m.p. of VI.

SALT LAKE CITY, UTAH

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[14] K. Fries and H. Hubner, *Ber.*, **39**, 439 (1906).

[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

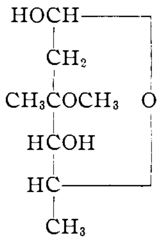
Erythromycin. VII. The Structure of Cladinose[1]

BY PAUL F. WILEY AND OLLIDENE WEAVER

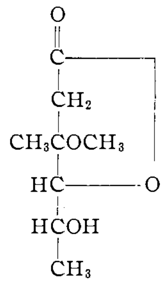
RECEIVED AUGUST 26, 1955

Cladinose, the nitrogen-free sugar from erythromycin, has been degraded to acetaldehyde and β-formylcrotonic acid, thus showing cladinose to have structure I.

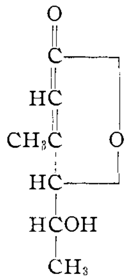
The isolation of cladinose from erythromycin has been reported previously.[2,3] Cladinose was shown to have the composition $C_8H_{16}O_4$ and to contain two C–CH_3 groups, two hydroxyl groups and one methoxyl group. Evidence was presented which showed the presence of a hemiacetal grouping and the moiety CH_3CHO– with participation of this oxygen in the hemiacetal ring. These facts and material presented in this paper prove that cladinose has structure I.[4]



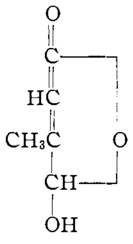
I



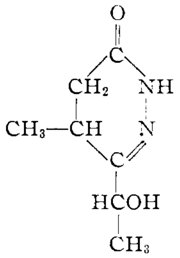
II



III



IV



V

Oxidation of cladinose with aqueous bromine gave rise to a lactone II. This compound was never obtained pure as it lost the methoxyl group on distillation. However, the infrared spectrum of II showed absorption at 2.90 and 5.65 μ with a shoulder at 5.81 μ which is consistent with a hydroxy-γ-lactone having a smaller amount of a δ-lactone present. The lactone was characterized as its 3,5-dinitrobenzoate which was a derivative of a γ-lactone (absorption at 5.63 μ in the infrared).

Treatment of II with 5% sodium hydroxide solution followed by neutralization eliminated the methoxyl group and formed an unsaturated lactone III. This lactone was not completely purified due to decomposition on distillation nor was a completely unambiguous derivative formed, but there is considerable evidence to indicate structure III. The impure product had absorption in the ultraviolet at 212 mμ, ε 10,000, consistent with an α,β-unsaturated lactone. The infrared absorption curve showed hydroxyl absorption at 3.0 μ, mixed γ- and δ-lactone absorption at 5.6–5.8 μ and >C=C< absorption at 6.1 μ. Methoxyl analysis indicated the absence of such a group. Reaction of the unsaturated lactone with hydrazine formed a crystalline derivative which has the composition $C_7H_{12}N_2O_2$. It was concluded that this product has structure V because of its analysis and the great similarity of its ultraviolet spectrum to that of 4,5-dihydro-6-methyl-3(2H)-pyridazone. Both compounds absorbed at 242 mμ with V having ε 7520

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[1] A preliminary report of this work was published as a Communication to the Editor: see P. F. Wiley and O. Weaver, THIS JOURNAL, **77**, 3422 (1955).

[2] E. H. Flynn, M. V. Sigal, Jr., P. F. Wiley and K. Gerzon, *ibid.*, **76**, 3121 (1954).

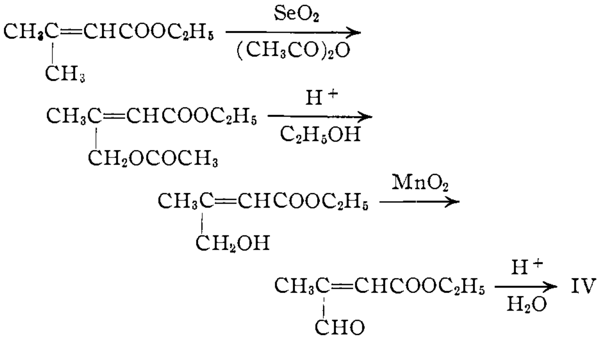
[3] R. B. Hasbrouck and F. C. Garven, *Antibiotics & Chemotherapy*, **3**, 1040 (1953).

[4] No indication of absolute or relative configurations is intended in the formulas.

and 4,5-dihydro-6-methyl-3(2H)-pyridazone having ε 7170.

Periodate oxidation of cladinonic acid lactone (II), after opening the lactone ring with base, gave rise to two products. One of these was identified as acetaldehyde by conversion to its 2,4-dinitrophenylhydrazone. The second was shown to be β-formylcrotonic acid (apparently existing in its pseudo-acid form, IV) by conversion to its 2,4-dinitrophenylhydrazone and semicarbazone and comparison of these with synthetic samples. β-Formylcrotonic acid was not isolated in a pure state due to its instability during distillation. The structure IV seems likely on the basis of the infrared spectrum of the impure material which has absorption at 3.0 μ indicating a hydroxyl group, absorption at 5.70 μ indicating a γ-lactone and absorption at 6.08 μ indicating olefinic unsaturation. Rinkes[5] has reported the isolation of β-formylcrotonic acid from the degradation of methylbixin. However, he reported that his compound formed a semicarbazone melting at 257° while our semicarbazone melted at 217°, so the compounds are probably not identical.

The synthesis of β-formylcrotonic acid was achieved by the following route.



$$\begin{array}{l} CH_3C{=}CHCOOC_2H_5 \xrightarrow[(CH_3CO)_2O]{SeO_2} \\ \;| \\ CH_3 \end{array}$$

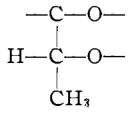
$$\begin{array}{l} CH_3C{=}CHCOOC_2H_5 \xrightarrow[C_2H_5OH]{H^+} \\ \;| \\ CH_2OCOCH_3 \end{array}$$

$$\begin{array}{l} CH_3C{=}CHCOOC_2H_5 \xrightarrow{MnO_2} \\ \;| \\ CH_2OH \end{array}$$

$$\begin{array}{l} CH_3C{=}CHCOOC_2H_5 \xrightarrow[H_2O]{H^+} IV \\ \;| \\ CHO \end{array}$$

Ethyl β-hydroxymethylcrotonate was prepared according to the method of Sobotka and Rubin.[6] Oxidation to the aldehyde ester was done using the procedure of Attenburrow and co-workers.[7] Attempted purification of synthetic IV by distillation was unsuccessful. However, the crude product had an infrared absorption spectrum with a hydroxyl band at 3.0 μ, a carbonyl band at 5.70 μ indicating a γ-lactone and a >C=C< band at 6.05 μ. This is consistent with structure IV and agrees well with the absorption spectrum of IV as obtained from cladinose. Synthetic IV was converted to a 2,4-dinitrophenylhydrazone and a semicarbazone, both of which were easily purified.

The oxidation of cladinose to a lactone is further confirmation of the aldose structure for cladinose and places one hydroxyl group at C-1. Although it was previously reported[2,3] that cladinose is not oxidized by sodium metaperiodate, this does not necessarily prove the absence of adjacent hydroxyl groups.[8] In fact the 1,2-glycol grouping has been shown to be present in cladinose by oxidation with periodate of base-opened cladinonic acid lactone to give acetaldehyde and β-formylcrotonic acid. The positive iodoform reaction of cladinose and the isolation of acetaldehyde after periodate oxidation of cladinose lactone prove the presence of the grouping



$$\begin{array}{c} -C-O- \\ | \\ H-C-O- \\ | \\ CH_3 \end{array}$$

in cladinose and its lactone. The isolation of β-formylcrotonic acid and acetaldehyde prove the presence of a six-carbon chain in cladinose. From these facts it is evident that the above moiety represents C-4, C-5 and C-6 of cladinose. It has been shown already[2] that the hetero oxygen atom of cladinose is on the carbon atom that has now been shown to be C-5. Therefore the second hydroxyl group in cladinose is at C-4 and cladinose has a pyranose ring. The formation of β-formylcrotonic acid also shows that the second C-CH$_3$ group is at C-3 in cladinose. Both the formation of III and IV after base treatment of II is indicative that the methoxyl group in cladinonic acid lactone is labile to base and must, therefore, be β to the carbonyl group and at C-3 in cladinose. Thus, the structure of cladinose is necessarily I.

Acknowledgment.—The authors wish to thank Messrs. W. L. Brown, H. L. Hunter and G. M. Maciak and Miss Gloria Beckmann for microanalyses; and Drs. H. Boaz and H. A. Rose and Mr. J. Forbes for physical chemical data. We also wish to thank Drs. E. H. Flynn, M. V. Sigal, Jr., and K. Gerzon for helpful advice.

Experimental[9]

Cladinonic Acid Lactone (II).—Twelve and eight-tenths grams (0.08 mole) of bromine was added to a solution of 7.0 g. (0.04 mole) of cladinose in 210 ml. of water. The mixture was shaken until all the bromine had dissolved. The resulting solution was allowed to stand in the dark for seven days. The excess bromine was removed by evaporation under reduced pressure at room temperature. The aqueous residue was filtered, and the filtrate was extracted continuously with benzene for 48 hours. Evaporation of the benzene extract under reduced pressure at room temperature left a light brown liquid residue weighing 5.15 g. Distillation of this material gave a product boiling at a bath temperature of 120–130° at 0.2 mm. However, the methoxyl analysis indicated loss of a considerable portion of the methoxyl groups. The ultraviolet spectrum of the undistilled material showed only end absorption. The infrared spectrum of the same material had strong absorption at 2.90 and 5.65 μ with a shoulder at 5.81 μ.

Cladinonic Acid Lactone 3,5-Dinitrobenzoate.—This 3,5-dinitrobenzoate was prepared according to Brewster's procedure.[10] One and three-tenths grams (5.8 mmoles) of 3,5-dinitrobenzoic acid was added to 6 ml. of dry pyridine and 2.2 g. (11.6 mmoles) of *p*-toluenesulfonyl chloride was added to the pyridine solution. The mixture evolved heat and solution occurred. This solution was cooled in an ice-bath and added to 1.0 g. (5.8 mmoles) of cladinonic acid lactone. The reaction mixture was cooled in an ice-bath for one hour and poured with vigorous stirring into 18 ml. of ice-water. This mixture was stirred, thoroughly, and the

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[5] I. J. Rinkes, *Rec. trav. chim.*, **48**, 1093 (1929).

[6] H. H. Sobotka and M. I. Rubin, U. S. Patent 2,390,335 (1945).

[7] J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Herns, A. B. A. Jansen and F. Walker, *J. Chem. Soc.*, 1094 (1952).

[8] H. Klosterman and F. Smith, THIS JOURNAL, **74**, 5336 (1952).

[9] Melting points and boiling points are uncorrected. Infrared spectra were obtained with a Baird double-beam recording spectrophotometer. Ultraviolet measurements were made with a Cary recording spectrophotometer.

[10] J. H. Brewster and C. J. Ciotti, Abstracts of Papers of September, 1955, Meeting of the American Chemical Society, p. 60-O.

water was removed by decantation. The product was washed twice more with water in the same fashion. The crystalline solid was recrystallized twice from alcohol with charcoal treatment and three more times without charcoaling. The final product melted at 123–125°, yield 0.40 g. (19%). The infrared spectrum of this product had peaks at 5.63 and 5.80 μ with no absorption in the hydroxyl region.

Anal. Calcd. for $C_{15}H_{16}N_2O_9$: C, 48.91; H, 4.35; N, 7.62; CH_3O(1), 8.42. Found: C, 48.85; H, 4.33; N, 7.64; CH_3O, 8.71.

Unsaturated Lactone III from Base-treated Cladinonic Acid Lactone.—Seven grams of cladinonic acid lactone was dissolved in 140 ml. of 5% sodium hydroxide solution. The resulting solution was allowed to stand overnight. Concentrated hydrochloric acid was added until a *p*H of 2.0 was reached. The acid solution was evaporated to dryness under reduced pressure. The residue was extracted with three 45-ml. portions of hot, dry acetone filtering each extract. The combined extracts were evaporated to dryness, and the residue was extracted again with two 20-ml. portions of cold, dry acetone filtering each extract. The combined extracts were evaporated to dryness leaving 5.1 g. of a brown liquid. This liquid was distilled under reduced pressure. No constant boiling fraction was obtained so the fraction boiling at 117 to 130° at 0.7 mm. was retained. This fraction had $n^{25}D$ 1.4849. The ultraviolet absorption spectrum had a peak at 212 mμ, ϵ 10,000. The infrared spectrum had a peak at 3.0 μ, a broad peak at 5.6 to 5.8 μ and a peak at 6.1 μ.

This experiment is an example of numerous attempts to obtain pure III. Analyses on these samples gave carbon values 1–2% lower than the theoretical and hydrogen values 0.15–0.40% higher than theoretical.

Hydrazine Derivative of III.—One gram of III was dissolved in 4 ml. of methanol, and 12 drops of hydrazine was added. The solution was refluxed for 18 hours. The solvent was removed by evaporation under reduced pressure leaving a solid residue. Recrystallization of the solid from alcohol gave 0.45 g. (50%) of crystalline solid, m.p. 188–191°. Two more recrystallizations from alcohol gave a product melting at 190–192°. The ultraviolet spectrum had an absorption peak at 242 mμ, ϵ 7520.

Anal. Calcd. for $C_7H_{12}N_2O_2$: C, 53.84; H, 7.68; N, 17.94; mol. wt., 156. Found: C, 53.91; H, 7.70; N, 17.66; mol. wt., 192.

Periodate Oxidation of Cladinonic Acid Lactone. (a) **Isolation of Acetaldehyde.**—Fifty-five hundredths gram (3.2 mmoles) of cladinonic acid lactone was dissolved in 15 ml. of 10% sodium hydroxide solution. After the solution had stood overnight it was neutralized to *p*H 6.7 using 1.0 N hydrochloric acid. Sixty-eight hundredths gram (32 mmoles) of sodium metaperiodate was added and the solution was allowed to stand at room temperature for 24 hours. The solid precipitate was removed by filtration. The filtrate was extracted with six 10-ml. portions of benzene. The extracts were added to 650 ml. of Brady reagent. After this mixture had stood overnight, the benzene was removed by evaporation and the residue was filtered. The solid product was recrystallized four times from alcohol. It then melted at 165–167° and gave no depression in melting point when it was mixed with an authentic sample of acetaldehyde 2,4-dinitrophenylhydrazone. An X-ray diffraction pattern was identical with that of authentic acetaldehyde 2,4-dinitrophenylhydrazone.

(b) Isolation of β-Formylcrotonic Acid (IV).—Four grams (0.023 mole) of cladinonic acid lactone was dissolved in 100 ml. of water. A solution of 2.0 *N* sodium hydroxide was added until the *p*H of the solution was stable at *p*H 11.8. The solution was then adjusted to *p*H 7.9 with concentrated hydrochloric acid, and 4.9 g. (0.023 mole) of sodium metaperiodate was added. After the solution had stood at room temperature for three days, it was filtered and evaporated to dryness under reduced pressure at room temperature. The residue was extracted with three 20-ml. portions of hot, dry acetone filtering each extract. Evaporation of the combined extracts left 3.2 g. of liquid residue. Attempts to purify this material by distillation were unsuccessful. The infrared absorption spectrum had peaks at 3.0, 5.70 and 6.08 μ.

Two-tenths gram of this product was converted to the 2,4-dinitrophenylhydrazone by the method of Shriner and Fuson.[11] Three recrystallizations from acetone gave a product melting at 250–251° dec.

Anal. Calcd. for $C_{11}H_{10}N_4O_6$: C, 44.90; H, 3.43; N, 19.03; mol. wt., 294. Found: C, 45.15; H, 3.68; N, 18.86; mol. wt., 290.

A second two-tenths of a gram was converted to its semicarbazone by the method of Shriner and Fuson.[11] After this product had been recrystallized three times from an ethanol–water mixture, it melted at 214–215° dec.

Anal. Calcd. for $C_6H_9N_3O_3$: C, 42.11; H, 5.26; N, 24.56. Found: C, 42.42; H, 5.36; N, 24.30.

Ethyl β-Formylcrotonate.—A mixture of 6.4 g. of ethyl β-hydroxymethylcrotonate, 40 g. of specially prepared manganese dioxide[7] and 320 ml. of petroleum ether (b.p. 60–70°) was stirred for 3.5 hours. The mixture was filtered, and the filter cake was washed with a little petroleum ether. The filtrate was concentrated on the steam-bath until all the solvent was removed. The residue was dissolved in 30 ml. of chloroform, and the solution was filtered and again concentrated. Distillation of this residue gave 1.5 g. of product, b.p. 51° at 2.0 mm. This product was fractionated and a middle cut, $n^{25}D$ 1.4569, was submitted for analysis. The ultraviolet spectrum showed strong absorption at 220 mμ, ϵ 12,300. The infrared spectrum had peaks at 3.55, 3.68, 5.82, 5.90 and 6.08 μ.

Anal. Calcd. for $C_7H_{10}O_3$: C, 59.15; H, 7.04; C_2H_5O (1), 31.70; mol. wt., 142. Found: C, 58.91; H, 7.31; C_2H_5O, 32.55; mol. wt., 153.

β-Formylcrotonic Acid.—One and one-half grams of ethyl β-formylcrotonate was dissolved in 5 ml. of 6 *N* hydrochloric acid, and the solution was refluxed for two hours. The solution was evaporated to dryness under reduced pressure. The residue was dissolved in water and evaporated to dryness. Repetition of the solution in water and evaporation gave a liquid, wt. 1.1 g. The infrared spectrum had peaks at 3.0, 5.70 and 6.05 μ.

A sample of this was converted to its 2,4-dinitrophenylhydrazone by the method of Shriner and Fuson.[11] The derivative was recrystallized twice from acetone giving a product melting at 251° dec. This product did not depress the melting point of the 2,4-dinitrophenylhydrazone of β-formylcrotonic acid derived from cladinose when the two were mixed, and the infrared spectra of the two samples were identical.

Anal. Calcd. for $C_{11}H_{10}N_4O_6$: C, 44.90; H, 3.43; N, 19.03. Found: C, 45.06; H, 3.55; N, 18.89.

Another sample of β-formylcrotonic acid was converted to its semicarbazone by the method of Shriner and Fuson.[11] The semicarbazone melted at 217° dec. after it had been recrystallized four times from ethanol. The X-ray diffraction patterns, the infrared spectra and the ultraviolet spectra of this product and of the semicarbazone of β-formylcrotonic acid derived from cladinose were identical.

Anal. Calcd. for $C_6H_9N_3O_3$: C, 42.11; H, 5.26; N, 24.56. Found: C, 42.13; H, 5.38; N, 24.30.

INDIANAPOLIS, INDIANA

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[11] R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1935.